

In the Claims:

Please cancel Claims 1-40 and add new Claims 41-87 as follows:

Sub B1
41. A method for determining sequence of a nucleic acid molecule, comprising the steps of:

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a) synthesizing a nucleic acid molecule de novo from a RNAP promoter sequence in a reaction mixture containing a mutant T7-type RNA polymerase in each of four separate reactions, wherein said mutant T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydroxy or a hydrogen or a fluorine at the 2'-position, and further comprising a ddNTP, such that each of the four separate reactions forms a plurality of reaction products of differing length, the length of said reaction products indicating the positions or the type of base corresponding to the incorporated ddNTP, and
b) evaluating the reaction products so that the sequence of the template molecule may be deduced.

42. The method of Claim 41, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gnl, Y and A1122.

2 43. The method of Claim 41, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP.

3 44. The method of Claim 41, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, and 5-hydroxy-methyl-dCTP.

4 45. The method of Claim 41, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 2'-deaza-2'-F-GTP, 2'-F-ITP, 5-methyl-2'-F-CTP, and 5-hydroxymethyl-2'-F-CTP.

5 46. A method for determining the sequence of a nucleic acid molecule, comprising the steps of:

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a) synthesizing a nucleic acid molecule by extending a primer, wherein at least part of the primer is complementary to a template molecule so as to anneal therewith, in a reaction mixture containing a mutant T7-type RNA polymerase in each of four separate reactions, wherein said mutant T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, each comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydroxy or a hydrogen or a fluorine at the 2'-position, and further comprising a ddNTP, such that each of the four separate reactions forms a plurality of reaction products of differing length, the length of said reaction products indicating the positions of the type of base corresponding to the incorporated ddNTP; and

b) evaluating the reaction products so that the sequence of the template molecule may be deduced.

6 47. The method of Claim 46, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

7 48. The method of Claim 46, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP.

8 49. The method of Claim 46, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, and 5-hydroxymethyl-dCTP.

9 50. The method of Claim 46, wherein each reaction comprises at least four nucleoside triphosphates chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 2'-deaza-2'-F-GTP, 2'-F-ITP, 5-methyl-2'-F-CTP, and 5-hydroxymethyl-2'-F-CTP.

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51. The method of Claim 41, wherein a dinucleotide or trinucleotide for initiation of de novo acid synthesis is added to the reaction mixture.

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52. The method of Claim 41, wherein at least one of the nucleoside triphosphates in the reaction mixture is modified to contain a radioactive or non-radioactive label.

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53. The method of Claim 41, wherein the ddNTP in the reaction mixture is modified to contain a radioactive or non-radioactive label.

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54. The method of Claim 51, wherein the dinucleotide or trinucleotide in the reaction mixture is modified to contain a radioactive or non-radioactive label.

Sub B2
55. A method for determining the sequence of a nucleic acid molecule, comprising the steps of:

a) synthesizing a nucleic acid molecule de novo from a RNAP promoter sequence in a reaction mixture containing a mutant T7-type RNA polymerase in each of four separate reactions, wherein said mutant T7-type RNA polymerase has a reduced, discrimination between canonical and non-canonical nucleoside triphosphates, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydrogen or a fluorine at the 3'-position, and further comprising a rNTP;

b) treating the nucleic acid products of the reactions so as to bring about hydrolysis of the rNTP has been incorporated, whereby a plurality of reaction products of differing length are formed, the length of said reaction products indicating the positions of the type of base corresponding to the incorporated rNTP; and

c) evaluating the reaction products so that the sequence of the template molecule may be deduced.

56. The method of Claim 55, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

15 51. The method of Claim 55, wherein the nucleic acid synthesis is part of or coupled to a method for nucleic acid amplification.

58. A kit for performing the method of Claim 55 comprising a mutant T7-type RNA polymerase, wherein said mutant T7-type RNA polymerase has a reduced discrimination triphosphates and data or instructions describing conditions under which the method of Claim 55 may be performed.

59. A kit for performing the method of Claim 57 comprising a mutant T7-type RNA polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of Claim 57 may be performed.

16 60. A method for determining the sequence of a nucleic molecule, comprising the steps of:

a) synthesizing a nucleic acid molecule by extending a primer, wherein at least part of the primer is complementary to a template molecule so as to anneal therewith, in a reaction mixture containing a mutant T7-type RNA polymerase in each of four separate reactions, wherein said mutant T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, each reaction comprising at least four nucleoside triphosphates, wherein at least one nucleoside triphosphate has a nucleic acid base which is complementary to each of adenine, cytidine, guanine and uracil or thymine and a sugar with either a hydrogen or a fluorine at the 2'-position, and further comprising a rNTP;

b) treating the nucleic acid products of the reactions so as to bring about hydrolysis of the phosphodiester backbone at all sites where a rNTP has been incorporated, whereby a plurality of reaction products of differing length are formed, the length of said reaction products indicating the positions of the type of base corresponding to the incorporated rNTP; and

c) evaluating the reaction products using any of the methods common in the art for separating and detecting products of sequencing reactions so that the sequence of the template molecule may be deduced.

17 61. The method of Claim 16, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

18 62. The method of Claim 16, wherein the nucleic acid synthesis is part of or coupled to a method for nucleic acid amplification.

63. A kit for performing the method of Claim 60 comprising a mutant T7-type RNA polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of Claim 60 may be performed.

64. A kit for performing the method of Claim 62 comprising a mutant T7-type RNA polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or instructions describing conditions under which the method of Claim 62 may be performed.

65. A kit for performing a partial ribo-substitution reaction comprising a mutant T7-type RNA polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or information describing conditions under which the method may be performed.

66. A kit for determining a sequence of a nucleic acid molecule by a dideoxy sequencing procedure, which kit comprises:

- a) a mutant T7-type RNA polymerase, wherein said mutant T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates;
- b) four nucleoside triphosphates; and
- c) four ddNTPs.

67. The kit of Claim 66, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

68. The kit of Claim 66, wherein said nucleoside triphosphates are chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP and said ddNTPs are ddATP, ddCTP, ddGTP, and ddUTP or ddTTP.

69. The kit of Claim 66, wherein said nucleoside triphosphates are chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, and 5-hydroxy-methyl-dCTP and said ddNTPs are ddATP, ddCTP, ddGTP, and ddUTP or ddTTP.

70. The kit of Claim 66, wherein said nucleoside triphosphates are chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 2'-deaza-2'-F-GTP, 2'-F-ITP, 5-methyl-2'-F-CTP, and 5-hydroxymethyl-2'-F-CTP and said ddNTPs are ddATP, ddCTP, ddGTP, and ddUTP or ddTTP.

71. A kit for determining a sequence of a nucleic acid molecule by a dideoxy sequencing procedure, which kit comprises:

- a) a mutant T7-type RNA polymerase, wherein said mutant T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates;
- b) a primer for extending, in a template-dependent manner, a nucleic acid comprising a sequence complementary to said nucleic acid molecule;
- c) four nucleoside triphosphates; and
- d) four ddNTPs.

72. The kit of Claim 71, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

73. The kit of Claim 71, wherein said nucleoside triphosphates are chosen from the group consisting of ATP, CTP, GTP, and UTP or rTTP and said ddNTPs are ddATP, ddCTP, ddGTP, and ddUTP or ddTTP.

74. The kit of Claim 71, wherein said nucleoside triphosphates are chosen from the group consisting of dATP, dCTP, dGTP, dUTP, dTTP, 7-deaza-dGTP, dITP, 5-methyl-dCTP, and 5-hydroxy-methyl-dCTP and said ddNTPs are ddATP, ddCTP, ddGTP, and ddUTP or ddTTP.

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75. The kit of Claim 71, wherein said nucleoside triphosphates are chosen from the group consisting of 2'-F-ATP, 2'-F-CTP, 2'-F-GTP, 2'-F-UTP, 2'-F-TTP, 2'-deaza-2'-F-GTP, 2'-F-ITP, 5-methyl-2'-F-CTP, and 5-hydroxymethyl-2'-F-CTP and said ddNTPs are ddATP, ddCTP, ddGTP, and ddUTP or ddTTP.

76. A method for synthesizing a nucleic acid molecule comprising at least one non-canonical nucleotide, comprising the steps of:

a) incubating a template nucleic acid in a reaction mixture under nucleic acid synthesis conditions containing (i) a mutant T7-type RNA polymerase, wherein said T7-type RNA polymerase has a reduced discrimination between canonical and non-canonical nucleoside triphosphates, and (ii) at least one non-canonical nucleoside triphosphate, wherein said non-canonical nucleoside triphosphate is incorporated into the synthesized nucleic acid in place of only one canonical nucleoside triphosphate; and

b) obtaining the synthesis of a nucleic acid molecule comprising at least one non-canonical nucleotide.

77. The method of Claim 76, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.

78. The method of Claim 76 wherein the template nucleic acid is DNA.

79. The method of Claim 76 wherein the template nucleic acid is RNA.

80. The method of Claim 76 wherein a nucleic acid molecule comprising at least one non-canonical nucleotide is synthesized by extension of a primer molecule, at least part of which is sufficiently complementary to a portion of the template to hybridize therewith.

81. The method of Claim 76 wherein a nucleic acid molecule comprising at least one non-canonical nucleotide is synthesized *de novo* without using a primer molecule.

82. The method of Claim 76 wherein the non-canonical nucleoside triphosphate is a 2'-fluoro-nucleoside triphosphate.

83. The method of Claim 76 wherein the synthesized nucleic acid molecule has an altered susceptibility to a ribonuclease or a deoxyribonuclease compared to a nucleic acid which is synthesized using the corresponding non-mutant RNA polymerase.

84. The method of Claim 76 wherein the synthesized nucleic acid molecule is selected from the group consisting of a ribozyme or a nucleic acid molecule used for gene therapy, in a vaccine, in an antiviral composition, in an antimicrobial composition, in an antisense composition for regulating gene expression, in a composition for hybridization to a complementary nucleic acid, or as a probe for detection of a complementary nucleic acid.

85. The method of Claim 76 wherein the synthesized nucleic acid molecule is single-stranded.

86. A kit for performing the method of Claim 76, comprising a mutant T7-type RNA polymerase which has reduced discrimination between canonical and non-canonical nucleoside triphosphates and data or information describing conditions under which the method of Claim 76 may be performed.

87. The kit of Claim 86, wherein the T7-type RNA polymerase is selected from the group consisting of T3, ϕ I, ϕ IIH, W31, gh1, Y and A1122.